

INFLUENCE OF TEMPERATURE, SIZE AND SEX ON AERIAL RESPIRATION OF
POMACEA CANALICULATA (GASTROPODA: AMPULLARIIDAE)
FROM SOUTHERN PAMPAS, ARGENTINA

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INTRODUCTION

Pomacea canaliculata (Lamarck 1822) is a freshwater snail belonging to the family Ampullariidae, whose natural distribution comprises most of the Plata river Basin and extends southwards to Tandilia and Ventania mountains in southern Pampas, Buenos Aires Province, Argentina (Martín et al., 2001). International concern about this voracious macrophytophagous snail rose when it, and other related species, became established as a serious pest of aquatic crops, including rice and taro, in South and East Asia (Cowie et al., 2006). In addition, the role of invading ampullariids as promoters of ecosystemic changes in invaded natural wetlands has been recently highlighted (Carlsson et al., 2004). Nowadays, *P. canaliculata* is listed among the top 100 worst invasive alien species of the world (Lowe et al., 2000), being the only freshwater snail included and probably the species with the greater potential for spread in North America (Rawlings et al., 2007).

Ampullariids or apple snails have both a well-developed gill and a pulmonary sac or lung, and are commonly considered as “amphibious” snails (Andrews, 1965; Berthold, 1991). Water flows inside the pallial cavity through a furrow-shaped left nuchal lobe and leaves it through the right nuchal lobe. The lung is located in the left side of the mantle roof and opens anterolaterally to the pallial cavity. In *Pomacea*, during aerial respiration, the left nuchal lobe lengthens and rolls up to form a tube-shaped siphon, which conveys air into the lung, allowing the snails to ventilate it without reaching the water surface. During lung ventilation, pumping movements produced by the partial retraction of the cephalopodium generates a pressure differential leading to the exchange of air. The use of lung’s air as a physical gill has been denied (Andrews, 1965), although it increases apple snail’s buoyancy (Burky & Burky, 1977; Dillon, 2000).

Lung ventilation frequency in the few *Pomacea* species studied increases with water temperature (McClary, 1964) and decreases with body size, both in intra- and interspecific comparisons (Burky & Burky, 1977), but the basic parameters of aerial respiration are unknown for *P. canaliculata*. Most previous studies refer to species with tropical and subtropical ranges of distribution. However, *P. canaliculata* inhabits subtropical and temperate regions and remains active at low temperatures that are lethal or induce lethargy in the remaining species (Cowie, 2002). *Pomacea canaliculata* also shows considerable sexual dimorphism in such traits as shell and operculum shape (Estebenet et al., 2006), longevity, growth rate (Estebenet & Martín, 2002; Martín & Estebenet, 2002), and midgut gland size (Vega et al., 2005). These differences suggest the possibility of intersexual differences in metabolic rates and/or respiratory organs and hence on aerial respiratory behaviour.

Pomacea canaliculata deposit egg masses above the waterline, and the embryos fully develop inside the cleidoic eggs (Pizani et al., 2005). During development, the embryos are submersed in a perivitelline fluid providing them with structural precursors and energy supplies (Heras et al., 1998) until eggs hatch by mechanical fracture of the calcareous egg shell. It seems highly improbable that the embryos can fill the lungs except at the final stages of development when the perivitelline fluid has been totally incorporated. However, it is unknown whether lung ventilation begins just before falling from the egg mass or, alternatively, how long after the snails have fallen into the water.

Aerial respiration allows *Pomacea* spp. to thrive in freshwater habitats with poorly oxygenated waters and to endure dry-down conditions (Andrews, 1965; Cowie, 2002; Yusa et al., 2006). However, a number of drawbacks to

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the use of atmospheric oxygen has been suggested, such as limitations in distribution due to dependence on air access (Darby et al., 2002) and a higher vulnerability to specialized visual predators such as the snail kite *Rostrhamus sociabilis* (Vieillot, 1817) (Bennetts et al., 1994; Stevens et al., 2002). To better understand the role of aerial respiration on the ecology and behaviour of *P. canaliculata*, we analyzed the effect of temperature, size and sex on lung ventilation frequency and duration and number of pumping movements in this species.

MATERIALS AND METHODS

Snails used in this study were hatched from eggs laid in the laboratory by mature snails obtained in the summer 2004 from a population in Curamalal stream (37°14'31"S, 62°08'04"W, Buenos Aires Province, Argentina), a water-course originated in the Northern slope of Ventania Mountains. This stream section is narrow (3–8 m width) and shallow (mean depth less than 75 cm), although in some narrow places depth can reach up to 1.5 m; water velocities range from 0.39 to 0.93 m.s⁻¹ during normal discharge conditions. Patterns of daily and seasonal variation in water temperature in Curamalal stream were obtained from hourly readings of a submersible data-logger anchored to the bottom (1.2 m depth) during an entire year. The snails were maintained in aquaria with CaCO₃ saturated tap water in a rearing room at 25 ± 2°C, under 14 h light/day and fed with lettuce *ad libitum* (Martín & Estebenet, 2002).

Laboratory trials to estimate the parameters of aerial respiration were performed in 24 l glass aquaria (20 cm-wide, 40 cm-long and 30 cm-deep) with a sand layer deep enough to allow the snails to bury themselves (3 cm). Water temperatures (T) were kept constant at five different levels (10, 15, 20, 25 and 30°C), with electric thermostats located in the bottom of each aquarium to avoid thermal stratification. Aquarium water was continuously aerated with electric air pumps provided with diffusers in order to maintain dissolved oxygen at saturation levels. Snails used in the trials ranged from 13.5 to 47.0 mm of total shell length (L, from the apex to the extreme lip of the aperture, measured with a Vernier calliper to the nearest 0.1 mm). Snails in which the testicle was observed through the translucent shell (Takeda, 1999) or with a humped operculum (Estebenet et al., 2006) were considered

to be males. Snails without these male traits and smaller than 25 mm were considered as sexually undifferentiated, and those larger than that size as females, since a minimum of 25 mm has been considered necessary for maturation in females (Estebenet & Martín, 2002).

For each trial, six snails were selected to cover uniformly the whole size range and individually marked with synthetic polish. The snails were subjected to an acclimation period of 72–96 h, during which the water temperature was changed at a rate of 1°C per hour from 25°C to the desired one in the first hours; lettuce was supplied to each aquarium at a maximum rate of one leaf a day.

Snails were observed continuously for two hours in order to register respiratory activities. Thirty minutes before each observation period, lettuce remains were carefully removed and a new whole leaf was anchored to the bottom to avoid the continuous permanence of the snails at the waterline and to facilitate the register of siphonal movements. Just before each observation period water temperature was recorded and the air pumping system was disconnected to avoid interference with the observations. None of these procedures elicited negative responses (such as sinking, withdrawal, siphon retraction) in the snails. The starting and ending times and the number of pumping movements per lung ventilation during the observation period were recorded for each snail. The scheme was repeated three times for the 10°C and 15°C treatments and six times for the others. One hundred and forty-four snails were used in the trials, and each snail was used only once.

On the basis of the total records during the observation period the following variables were calculated: lung ventilation frequency (VF(h⁻¹) = number of lung ventilations / 2 h), number of pumping movements per lung ventilation (NP = number of pumping movements / number of lung ventilations) and duration of each pumping movement (DP(s) = time ventilating / number of pumping movements). Except for VF, only snails that ventilated at least once were included in the calculations.

Reliable measurements of siphon length proved to be impracticable due to its constant change in shape and position. As a surrogate, snail depth during lung ventilation was determined in a second group of trials, performed in the same 24 l aquaria with six snails of sizes ranging from 15.1 to 53.3 mm and an acclimation period of 24 h. In order to maximize lung

TABLE 1. Summary of two-way ANOVAs for the aerial respiration variables of *Pomacea canaliculata* (for differentiated snails only). Dependent variables (square root-transformed): lung ventilation frequency (VF), number of pumping movements per lung ventilation (NP) and duration of each pumping movement (DP); fixed factors: T (temperature), S (sex) and interaction (T x S).

Variables	MS error	Sex	Temperature	T x S
VF	0.5609	$F_{1,76} = 2.156$ $P = 0.1462$	$F_{4,76} = 32.081$ $p < 0.0001$	$F_{4,76} = 0.265$ $P = 0.8997$
NP	0.1535	$F_{1,50} = 0.014$ $P = 0.9060$	$F_{3,50} = 0.896$ $p = 0.4498$	$F_{3,50} = 1.289$ $P = 0.2883$
DP	0.0041	$F_{1,50} = 2.011$ $P = 0.1624$	$F_{3,50} = 145.147$ $p < 0.0001$	$F_{3,50} = 7.101$ $P < 0.0001$

ventilation frequency, water temperature was maintained at 30°C and no artificial aeration was provided. The distance from the water surface to the nearest point of the shell aperture at the beginning of a lung ventilation was recorded for 225 snails (those used in the previous trials plus an extra group from the same laboratory stock) while they were attached to the lateral walls of the aquarium; in most cases (93%) the depth of five to seven lung ventilations was registered for each snail, and maximum depth (MD (mm)) and average depth (AD (mm)) were calculated.

Eight hundred and seventy-two hatchlings obtained from four egg masses laid by laboratory snails on the aquarium walls were observed under a stereoscopic microscope at room temperatures (20–25°C) to investigate the presence of air in the lung and the respiratory behaviour. Ready to hatch eggs from the outer layer of the aerial egg masses were dissected with the same purpose, tearing apart the calcareous egg shell with a needle.

To test for the effect of temperature, sex and state of sexual differentiation over the aerial respiration variables, two-way ANOVAs were performed after a square root transformation as suggested by the Box-Cox method (Box & Cox, 1964). Analyses of differences among males, females and undifferentiated snails in the average and maximum distance during lung ventilation, were carried out with one-way ANCOVAs using length as covariate, after a log-log transformation.

RESULTS

Water temperatures in the Curamalal stream fluctuated greatly within the year, ranging from 31.5°C in January to a minimum of 0.3°C in July. Daily fluctuations were high, with a maximum recorded variation in one day of 17.8°C during December. Instantaneous temperature records showed nearly the same values along all depths.

TABLE 2. Summary of two-way ANOVAs for the aerial respiration variables of *Pomacea canaliculata* (for all snails; males and females not pooled for DP analysis). Dependent variables (square root-transformed): lung ventilation frequency (VF), number of pumping movements per lung ventilation (NP) and duration of each pumping movement (DP); fixed factors: T (temperature), D/S (state of differentiation or sex) and interaction (T x D/S).

Variables	MS error	Differentiation/Sex	Temperature	T x D/S
VF	0.5281	$F_{1,132} = 5.625$ $p = 0.0192$	$F_{4,132} = 38.782$ $p < 0.0001$	$F_{4,132} = 1.133$ $p = 0.3436$
NP	0.1670	$F_{1,84} = 17.246$ $p < 0.0001$	$F_{3,84} = 0.338$ $p = 0.7977$	$F_{3,84} = 1.634$ $p = 0.1877$
DP	0.0045	$F_{2,80} = 2.1002$ $p = 0.1291$	$F_{3,80} = 217.9807$ $p < 0.0001$	$F_{6,80} = 4.2099$ $p = 0.0009$

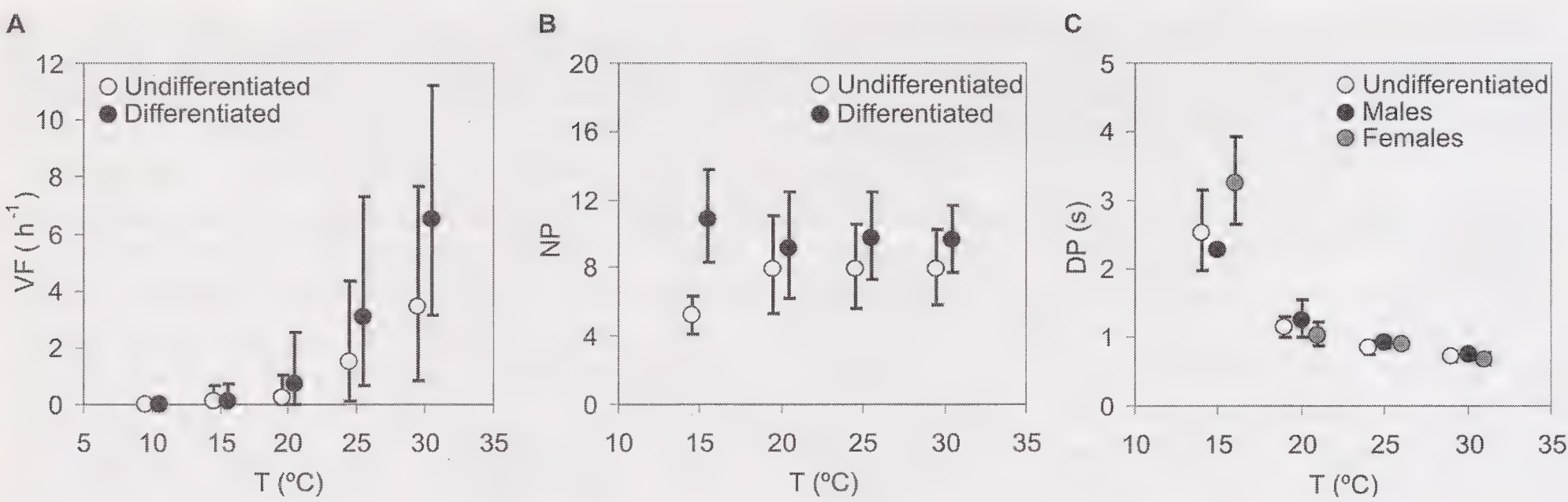


FIG. 1. Means $\pm$ SD (back-transformed) of aerial respiration variables of undifferentiated and differentiated *Pomacea canaliculata* snails vs. temperature (T): A) lung ventilation frequency (VF), B) number of pumping movements per lung ventilation (NP) and C) duration of each pumping movement (DP, males and females not pooled).

No significant differences were found between males ($n = 41$) and females ($n = 45$) for any of the aerial respiration variables (Table 1), although a significant interaction was detected for DP; accordingly, except for DP, both sexes were pooled in a single category (sexually differentiated) to compare them with the undifferentiated snails ($n = 56$) at the different temperatures tested. Lung ventilation frequency showed a positive significant effect of temperature and sexual differentiation; the undifferentiated snails ventilated significantly less often (64.78 to 47.71%) than differentiated ones at all temperatures above 15°C (Table 2, Fig. 1A). No snail ventilated its lung at 10°C and only 33% of them executed a few ventilations at 15°C. No effect of temperature but a significant one of sexual differentiation was detected on the number of pumping movements per lung ventilation (Fig. 1B); the general mean across all temperatures was significantly lower for

undifferentiated snails than for males and females (back-transformed means: 7.65 vs. 9.60, respectively). The duration of each pumping movement decreased significantly with temperature; the interaction with sex or differentiation was significant though lacked a clear pattern. No difference between males, females and undifferentiated snails was detected (Fig. 1C). For all snails on average, the duration of each pumping movement decreased abruptly from 2.69 s at 15°C to 1.15 s at 20°C and then declined gradually to 0.71 s at 30°C. As undifferentiated and differentiated snails differed not only in the degree of maturation but also in size, one-way ANCOVAs were performed to investigate whether an effect of size *per se* existed, using shell length as covariate (log-transformed). The group with the wider size range (sexually differentiated snails) and the temperature range where aerial respiration was more frequent (25–30°C) were

TABLE 3. Summary of one-way ANCOVAs of lung ventilation frequency (VF), number of pumping movements per lung ventilation (NP) and duration of each pumping movement (DP) (square root-transformed) of differentiated *Pomacea canaliculata* snails with shell length (L) as covariate (log-transformed); only the data for 25°C and 30°C were included in the analyses.

Variables	Slope equality	Common slope	Slope nullity	Intercept equality	Back-transformed adjusted means
VF	$F_{1,42} = 0.05$ $P = 0.8168$	4.6389	$F_{1,43} = 28.57$ $P < 0.0001$	$F_{1,43} = 19.89$ $P = 0.0001$	25°C: 2.92 30°C: 6.79
NP	$F_{1,39} = 0.53$ $P = 0.4704$	1.2687	$F_{1,40} = 7.99$ $P = 0.0073$	$F_{1,40} = 0.10$ $P = 0.7534$	25°C: 9.54 30°C: 9.74
DP	$F_{1,39} = 3.69$ $P = 0.0618$	-0.0869	$F_{1,40} = 2.35$ $P = 0.1330$	$F_{1,40} = 82.30$ $P < 0.0001$	25°C: 0.96 30°C: 0.84

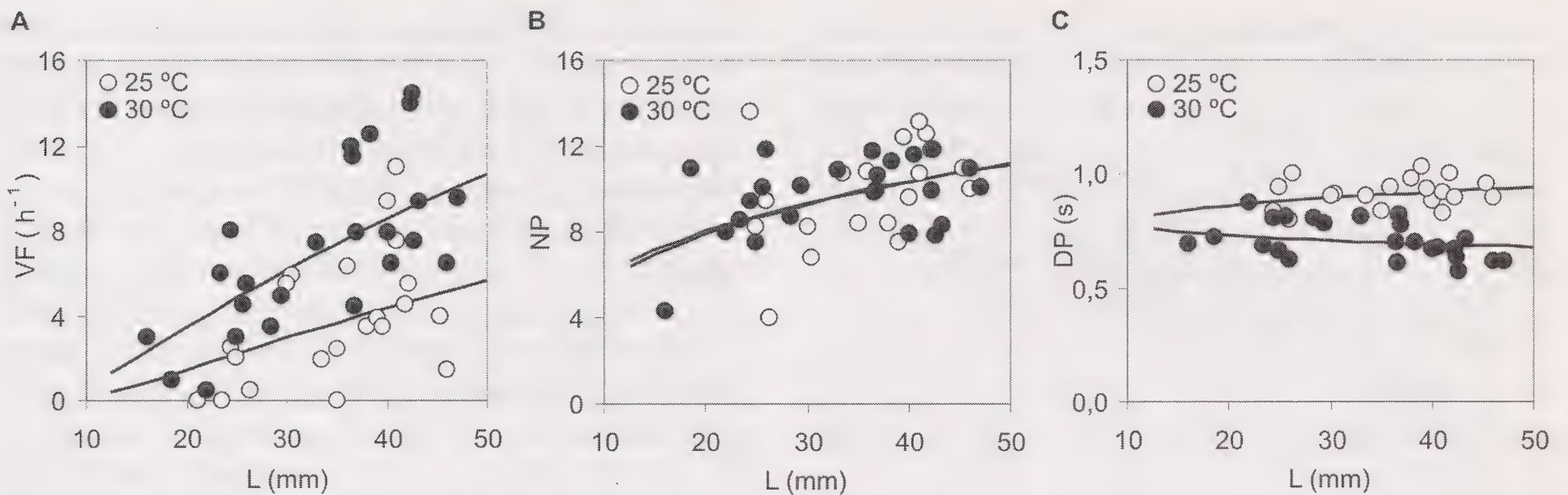


FIG. 2. Scatter plots of aerial respiration variables of differentiated *Pomacea canaliculata* snails vs. total shell length (L) at 25°C and 30°C: A) lung ventilation frequency (VF), B) number of pumping movements per lung ventilation (NP) and C) duration of each pumping movement (DP) (prediction curves of the aerial respiration variables (square root-transformed) vs. shell length (log-transformed) were estimated by least squares for each temperature).

selected for these analyses. Lung ventilation frequency increased significantly with shell length, the rates of increase (slopes) were not different at 30 and 25°C; the adjusted means (intercepts) were higher at 30°C (Table 3, Fig. 2A). The number of pumping movements also increased with snail size but, as pointed out in the ANOVA, no effect of temperature was evident (Table 3, Fig. 2B). The duration of each pumping movement was not affected by snail size (Table 3, Fig. 2C).

The slopes of the relationships of average and maximum depths with shell length were significantly higher for undifferentiated snails than for females and males (Table 4, Fig. 3). Males had slightly lower adjusted means for both average and maximum depths; a higher

variability for females was also apparent. The great dispersion of depths was mainly due to the different degree of extension, curvature, and direction of the siphon relative to shell position. In all events of aerial respiration observed in the two series of trials, the snails were adhered to the lateral walls of aquaria during the entire process of lung ventilation. No snails were observed spontaneously releasing their foothold to float to the surface.

Ninety-four percent of snails observed within three hours of hatching ($n = 872$) contained an air bubble in the lung. Lung ventilation consisted of the short extension of the left nuchal lobe, without forming a closed tube or siphon but folding it to form a furrow dorsally closed by the mantle edge; the air bubble was captured

TABLE 4. Summary of one-way ANCOVAs of average (AD) and maximum depth (MD) during lung ventilation of *Pomacea canaliculata* with shell length (L) as covariate (log-log-transformed) (slope equality was first checked for females, males and undifferentiated (F, M, U) snails, and then the analysis was continued with the groups with non-significantly different slopes (F, M)).

Variables	Slope equality (F, M, U)	Slopes	Slope equality (F, M)	Common slope	Slope nullity	Intercept equality	Adjusted means*
AD	$F_{2,208} = 12.44$ $P < 0.001$	F: 0.443 ^a M: 0.967 ^a U: 4.489 ^b	$F_{1,167} = 1.45$ $P = 0.2309$	0.7477	$F_{1,168} = 12.05$ $P < 0.001$	$F_{1,168} = 5.52$ $P < 0.02$	F: 15.038 M: 12.363
MD	$F_{2,208} = 10.16$ $P < 0.001$	F: 0.781 ^a M: 0.879 ^a U: 3.499 ^b	$F_{1,167} = 0.07$ $P = 0.7887$	0.8383	$F_{1,168} = 21.42$ $P < 0.001$	$F_{1,168} = 6.29$ $P < 0.02$	F: 23.408 M: 19.635

^{a,b} Different letters indicate significantly different slopes with Student t test ($p < 0.001$ after Dunn-Sidák correction)

*Back-transformed logarithmic adjusted means (mm)

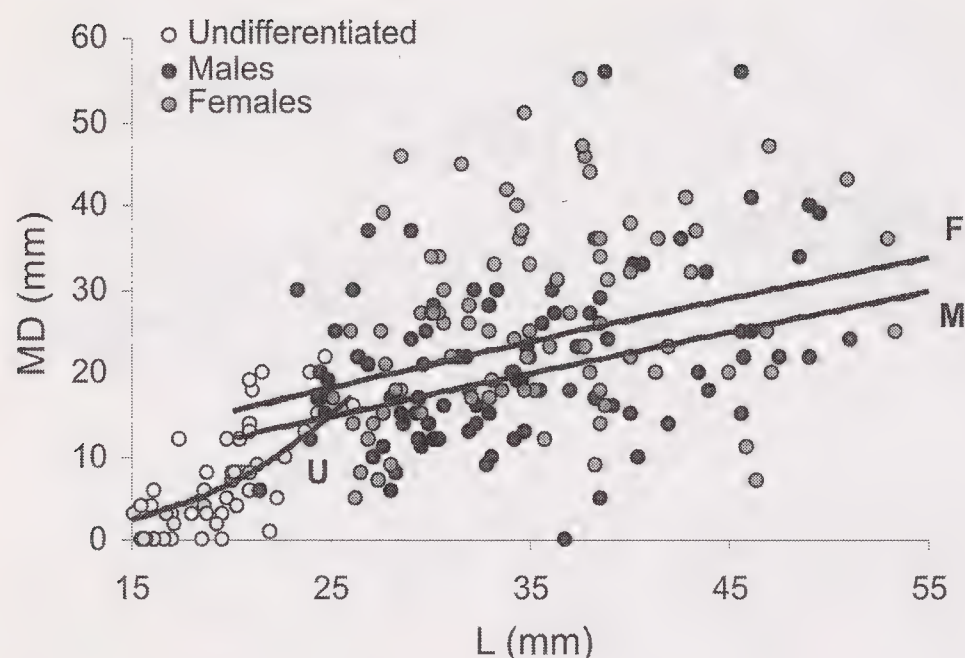


FIG. 3. Scatter plot of maximum depth during lung ventilation (MD) of *Pomacea canaliculata* vs. total shell length (L) (prediction curves were estimated by least squares after log-log transformation for female (F), male (M) and undifferentiated snails (U)).

in a single step, without any apparent pumping movement. The hatchlings usually take air while gliding on the water surface layer or when they first contact the surface after falling from the egg mass. In 95% of the cases, the snails removed from the calcareous shell of eggs in the last stage of development ($n = 114$) lacked an air bubble in the lung.

DISCUSSION

In *Pomacea canaliculata* aerial respiration begins within three hours of hatching (at 20–25°C) and is performed routinely as long as the snails are active; the lack of ventilation activity at 10°C was related to an almost total immobility of the snails since 94.5% remained fully retracted though not buried in the sand layer. In the southern Pampas, *P. canaliculata* remains in a lethargic state under rocks or among submersed plants for several months (Estebenet & Martín, 2002) although burying in mud to overwinter has also been suggested (Bachmann, 1960; Damborenea, 1996). *Pomacea paludosa* (Say 1829) fully retracts below 8°C, but remains immobile with the operculum slightly protruded beyond the shell aperture between 9°C and 13°C, probably to allow gill ventilation (Stevens et al., 2002). In the Curamalal stream even during the coldest months, part of the snails are found in the same state (unpublished data), indicating that eventually they open the operculum to allow some water circulation and they do not bury themselves; however they may end up buried

as a result of siltation. Although 94.5% of the snails were not retracted at 15°C, only a few ventilated the lung during the two hour observation period, indicating that the interval between ventilations is greater than two hours.

The range of temperatures used here (10–30°C) is narrower than the annual fluctuations recorded in Curamalal stream (0.3–31.5°C) but includes the whole range in which the snails are active in that water-body. According to water temperature records the ventilatory activity of *P. canaliculata* in Curamalal stream would be almost null between April and September, and hence its microdistribution would be independent of the accessibility to water surface. On the other hand, during Spring and Summer (between October and March) the higher temperatures would induce an increase in aerial respiration that could restrict snails' microdistribution due to the need of periodical access to air. Darby et al. (2002) showed that *Pomacea paludosa* avoids depths greater than 50 cm and suggested that it could be due to a combination of a difficulty in reaching the water surface to breathe and unsuitable substrate or food.

For an "amphibious" snail the effect of temperature on aerial respiration may be due to a combination of a decline in gill oxygen intake due to a decrease in dissolved oxygen and an increase in metabolic rate. In *P. canaliculata*, lung ventilation frequency increases 6.76-fold and the duration of each pumping movement decreases 0.63-fold between 20 and 30°C, resulting in a total 4.24-fold increase in the time devoted to lung ventilation. Although *Pomacea* spp. are able to regulate oxygen intake with decreasing dissolved oxygen (metabolic O_2 -regulators; Santos et al., 1987; Hanley & Ultsch, 1999), the small drop in that temperature range (17.6%; Margalef, 1983) seems not to be the main component of the increase in lung ventilation. On the other hand, the increase in specific oxygen consumption between 20 and 30°C for other *Pomacea* spp. (2.08 to 2.19-fold; Freiburg & Hazelwood, 1977; Santos et al., 1987) is also lower than the increase in the time devoted to lung ventilation in *P. canaliculata*. However, these rates belong to snails confined to small flasks and deprived of food so the response of activities that could contribute to raise metabolic rate (i.e., crawling and feeding) is very limited.

McClary (1964) registered for *P. paludosa* a 2.7-fold decrease and a 1.42 increase in the ventilation frequency for rapid changes (five minutes) in water temperature from 26 to 20°C and 26 to 30°C, respectively. For *P.*

canaliculata we estimated (for sexually differentiated snails after 72 h of acclimation) a 3.19-fold decrease for the range 25 to 20°C and 1.81-fold increase for 25 to 30°C. Perhaps the lower values in the former case indicate that the snails' bodies had not time enough to reach the water temperature during the one-hour period of observation. McClary (1964) also reported that lung ventilation increases under subsaturation levels of dissolved oxygen in *P. paludosa*; therefore, our observations of lung ventilation frequency should be considered as minima since they were made under conditions where aerial respiration is less promoted (i.e., dissolved oxygen near saturation).

Lung ventilation frequency showed a positive relationship with snail size (e.g.: 0.70 h⁻¹ to 4.35 h⁻¹ for 15 to 40 mm snails at 25°C, respectively). Assuming an isometric or simple scaling growth, the relative increase in ventilation frequency with snail size may be due to a decrease in the gas exchange surface relative to body mass (both in the gill and cutaneous surface). In fact, body mass grows even faster relative to shell length than expected by simple scaling (15.2%; Estebenet, 1998), probably indicating an increase in the relative fullness of the shell with flesh, since the overall shell shape changes little during growth (Estebenet et al., 2006). Contrarily to the relationship reported here, in the tropical apple snail *Pomacea urceus* (Müller 1774) lung ventilation frequency decreases with body size from 3.02 h⁻¹ for juvenile snails (22–32 mm) to 0.96 h⁻¹ for adult snails (90–125 mm) at 29°C (Burky & Burky, 1977). This dissimilar behaviour of ventilation frequency relative to size may be related to the fact that adult *P. urceus* aestivate buried in the mud during the dry season, displaying a set of reproductive, behavioural and physiological adaptations that includes the depression of the metabolic rate to one fifth of that of active snails (Burky et al., 1972).

The duration of each pumping movement decreased asymptotically with temperature, suggesting that the velocity of muscular contraction is the limiting factor in the response of this variable. The duration of each pumping movement at 25°C was of 0.89 s which compares well to the value of 0.9 s at 26°C registered in *P. paludosa* (McClary, 1964).

The number of pumping movements per ventilation was the only variable that showed no response to temperature but increased with size within sexually differentiated snails; size probably also explains the greater number of pumping movements of differentiated relative to

undifferentiated snails. The values for this variable ranged from 7.06 for 15 mm snails to 10.59 for 45 mm snails. Similarly, in *Pomacea urceus* the number of movements at 29°C ranged between 8 and 18 for snails of 22–32 mm and 90–125 mm, respectively (Burky & Burky, 1977); the number of pumping movements at 26°C for immature and mature individuals of *P. paludosa* were 15 to 18.7, respectively (McClary, 1964). The increase with size is probably related to an increase in the volume of the respiratory dead space, that is, the volume of air in the lung or the siphon not in direct contact with the gas exchange surface. Since the lung is a simple sac without internal folds (Andrews, 1965) and siphon length increases with snail size, a higher number of pumping movements is required to replace the air in the lung in large snails. In fact, a simple opening of the pneumostome without any pumping is apparently enough in the hatchlings of *P. canaliculata*.

The number of pumping movements observed here is close to those recorded in most species of similar adult size – 10.6 and 9.8 for *Pomacea falconensis* (Pain & Arias, 1958) and *Pomacea luteostoma* (Swainson, 1832), respectively (Burky & Burky, 1977). Although the values of 18.7 reported for adults of *P. paludosa* (McClary, 1964) are clearly higher, this may be related to differences in the relative siphon length or in lung volume.

Despite the noteworthy sexual dimorphism of *P. canaliculata* in several ecological and morphological traits (Estebenet & Martín, 2002; Vega et al., 2005; Estebenet et al., 2006), the aerial respiration variables investigated here showed no consistent differences between sexes. Likewise, oxygen consumption rates are similar for males and females in *P. paludosa* (Freiburg & Hazelwood, 1977) and *P. lineata* (Santos & Mendes, 1981). On the other hand, females ventilate the lung at greater depths than males, though this slight difference seems of little ecological significance. Perhaps this is a correlate of subtle differences in shape, curvature or length of the siphon. In females, the massive albumen gland bulges from the floor of the pallial cavity, occupying most of it (Andrews, 1965) and probably distorts the position of respiratory organs.

Two main roles have been assigned to the siphon in apple snails: to diminish their visibility to aerial predators during lung ventilation and to reach the water surface through dense mats of vegetation (Freiburg & Hazelwood, 1977). Paradoxically, *Pomacea* spp. have the longest siphon among neotropical genera of apple

snails (Andrews, 1965) and are the preferred prey of the snail kite *Rostrhamus sociabilis* (Snyder & Kale, 1983; Bennetts et al., 1994). Although the precise way in which the apple snails are detected is unknown, the extended siphon is probably the most visible part of the snail (Stevens et al., 2002), since shells are cryptic due to their colour, banding pattern and the presence of epibiontic algae. Nevertheless, since floating *P. canaliculata* snails are very rarely observed both in the laboratory and in the field, the success of a capture attempt depends on the ability to accurately locate and grab the shell beneath water surface. Although the entire range of depths recorded here (up to 6 cm) is well within the maximum potential reach of the snail kite (up to 16 cm; Sykes, 1987), the variability in siphon extension, curvature and direction could be critical to reduce the chance of an accurate localization in turbid water-bodies, which are the most frequently inhabited by *P. canaliculata* in the southern Pampas (Martín et al., 2001).

Independent of sexual differentiation, the depth during lung ventilation in *P. canaliculata* increases markedly beyond a shell length of approximately 20 mm. This would imply a heavier predation risk on small snails since they breathe air almost at the surface. However, *Pomacea* spp. smaller than 25 mm are rarely captured (e.g., Collett, 1977; Sykes, 1987; Tanaka et al., 2006), probably due to the combined effects of their low surfacing frequency (Bourne, 1993; our study), their low profitability (Beissinger et al., 1994), or a mechanical incapability of the snail kite talons to grasp snails below a certain diameter (Collett, 1977). The rapid increase in depth during lung ventilation could be interpreted as a behavioural response to counteract the raise in predation risk as snails grow beyond 20 mm.

Apple snails evolved from an aquatic ancestor with a ctenidium and developed a secondary lung inside the mantle cavity associated with an enlargement of shell aperture (Berthold, 1989). The other group of “amphibious” freshwater snails, the tiny pulmonate Basommatophora arose from a terrestrial lineage with a mantle cavity transformed into a lung and without a ctenidium (McMahon, 1983). According to Schmidt-Nielsen (1997), the interchange of air between the lung and the atmosphere occurs by ventilation only in vertebrates, though those of the siphonate apple snails (e.g., *Pomacea*, *Pila*, and *Marisa*) are clearly ventilation lungs

(Andrews, 1965; Burky & Burky, 1977; Freiburg & Hazelwood, 1977; Berthold, 1991; our study). In such small invertebrates as pulmonate snails, the interchange occurs by diffusion through the pneumostome (McMahon, 1983; Schmidt-Nielsen, 1997), as seems to happen in the hatchlings and small juveniles of *P. canaliculata* (our study) and also in adult apple snails without the left nuchal lobe developed into a siphon (e.g., *Saulea*, *Lanistes*; Berthold, 1991). Therefore, besides the ontogenetic effect of snail size on the number of pumping movements, the occurrence of ventilation in snails with a lung seems to be related to the presence of a siphon and hence to the need of renewing the air from the increased respiratory dead space.

Our study showed that even when food is available only at the bottom and dissolved oxygen is near saturation, *P. canaliculata* snails routinely move to the water surface to ventilate the lung, despite of the reduction in the time and energy allocated to other activities and the potential increase in predation risk. This continuous crawling from the bottom to the surface seems to be a common feature of the behaviour of *Pomacea* spp. (McClary, 1964; Burky & Burky, 1977; Freiburg & Hazelwood, 1977), especially in habitats with warm and poorly oxygenated waters. Furthermore, McClary (1964) reported that *P. paludosa* snails, even under dissolved oxygen saturation, tend to crowd under barriers that impede their access to the surface. The basis of the need for a periodical ventilation of the lung and the acute and chronic consequences of a prolonged lack of access to air in *P. canaliculata* are both unknown. The mechanism and degree of air dependence need to be clarified to reach a better comprehension of the effects of lung ventilation on distribution at different spatial scales and also on the time allocation relative to other routine activities.

ACKNOWLEDGEMENTS

This work was funded with grants by CONICET (Consejo Nacional de Investigaciones Científicas y Técnicas, PIP: 6150) and UNS (Universidad Nacional del Sur, PGI: 24B/108). MES is a doctoral fellow in CONICET. PRM is a researcher in CONICET. We are grateful to Nicolás Tamburi for providing the snails used in the trials.

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Revised ms. accepted 13 May 2008